# Ultrastructure and Organization of Gymnosperm Cell Walls

**H. HARADA**

*Wood Technology Institute, University of Kyoto*

The three important areas of investigation concerning the mature cell wall of gymnosperms are as follows: (1) the distribution of the principal chemical components in cell walls; (2) the physical nature of the structural units—cellulose micelles or microfibrils—also one of the most important chemical components of cell wall; and (3) the orientation of structural units (microfibrils) in the primary and secondary cell walls, with respect to the morphological cell axis. The first two areas will be treated by other authors in this volume. This paper is concerned with the third, namely, the physical organization of the cell wall structural units, and is based on the results of electron microscopic observations.

## Microfibrils as Structural Units

It is well known that the framework substance of wood cell walls consists of cellulose, while their matrix and incrusting substances consist of hemicellulose and noncellulosic substances such as lignin. It was discovered by applying electron microscopy that the cellulose molecules, which are the main constituents of the cell walls of native fibers, including wood cells, are aggregated in the form of microfibrils, thus forming independent structural units (Williams and Wyckoff, 1946). The width of microfibrils is 100 to 300 Å, varying with the kind of native fiber, and their length is indefinite (Mühlethaler, 1960). These results were obtained not only from the examination of samples of disintegrated wood cells, but also from the observations of replicas and ultrathin sections of the secondary wall of untreated tracheids and fibers (Hodge and Wardrop, 1950; Kobayashi and Utsumi, 1955; Jayme and Koburg, 1959; Côté and Day, 1962). The reason why the width of microfibrils is not agreed upon, but is subject to various opinions, is that the measurements by means of electron microscopy have been compared with the results of X-ray diffraction, which is the case for ramie. The width of the micelles (the crystalline

region) of ramie, obtained by the X-ray diffraction method, is 50 to 70 Å, and their length 600 Å (Hengstenberg and Mark, 1928). According to one opinion, the width of microfibrils is 50 to 100 Å, being equal to that of the micelle dimensions obtained by X-ray diffraction (Hodge and Wardrop, 1950; Rånby, 1958; Kobayashi and Utsumi, 1955). According to another, a microfibril is composed of 2 to 4 aggregated micellar strands forming an independent unit (Frey-Wyssling *et al.*, 1948). It is also said that the size of microfibrils is variable depending on the source of native fiber specimens (Wardrop, 1954; Mühlethaler, 1960). At present, however, it is generally supposed that in their natural state the structural units observed by electron microscopy occur as shown in the models proposed by Frey-Wyssling (1954, 1955). According to this concept, the microfibrils are about 200 Å in width and are further divided into smaller, elementary fibrils or micellar strands. Whether the structural units of wood cells are microfibrils or micellar strands, independent units in the form of fibrils can be observed with the aid of the electron microscope. Therefore, in this paper these units are called microfibrils.

## CELL WALL ORGANIZATION OF TRACHEIDS

What is the nature and composition of the cell wall? How are the microfibrils in the walls and layers oriented with respect to the tracheid axis? These problems will be dealt with in relation to the results of investigation by optical microscopy and X-ray diffraction, but will be limited to normal, mature gymnosperm tracheids.

The optical microscopic investigations of Kerr and Bailey (1934) first gave an established idea of the cell wall organization of the normal tracheids. They divided a cell wall, according to the order of its formation, into several layers of different nature: into the thin primary wall adjoining the intercellular layer and the thicker secondary wall, the latter being further divided into the outer, the central, and the inner layers, of which the central is thick, and the other two are thin. This division is due to the difference in the optical properties observed by polarizing microscopy in the transverse sections of tracheids. Between crossed nicols of the polarizing microscope the primary wall is slightly birefringent (shimmers dimly), the outer and the inner layers of the secondary wall shine brightly, and the central layer is extinct. This was considered to be due to the difference of the orientation of micelles in the primary wall and in each of the layers of the secondary wall. The organization of cell walls, thus conceived by Kerr and Bailey (1934), is frequently referred to even in electron microscopic investigations. The discussion in the present paper also starts from this conception.

Following Kerr and Bailey, many investigations to clarify the micellar orientation in the cell wall with the optical microscope and X-ray diffraction were reported: Bailey and Kerr, 1935; Preston, 1934; Onaka, 1936; Bailey and Vestal, 1937a, b; Ohara, 1939; Kubo and Go, 1941; Wardrop and Preston, 1947; Preston, 1952; Onaka and Harada, 1951; Harada *et al.*, 1951; and Bucher, 1957.

When electron microscopy was applied to the investigation of the tracheid wall organization, the first intention was to confirm, by direct observations, the conception established by Kerr and Bailey: namely, to distinguish the primary wall and the three layers of the secondary from one another, taking notice of their thickness, and to ascertain the microfibrillar orientation in each of them. It was first expected that this purpose would be accomplished by getting transverse sections of tracheids, as in the case of the optical microscopic investigations, and observing them with the aid of the electron microscope. However, this proved to be impossible because it was very difficult to get ultrathin sections from wood, and because the contrast of the ultrathin sections in the electron micrographs was not adequate. Therefore, instead of ultrathin sections, replicas of transverse sections of tracheids cut with an ordinary microtome were used, but the fine structure of the cell wall was masked by knife marks. Then the disintegration method was employed and the orientation of microfibrils in the primary wall could be observed in the disintegrated specimens of cambial cells, for instance. In addition to this method, the replication method was applied. If the surfaces of macerated tracheids were replicated, the structure of the primary wall could be seen; and because the tracheid wall has, as stated below, a lamellar structure, replicas of the split sections of a cell wall showed the structure of a layer or a lamella of the wall. If in this case several layers or lamellae, different in structure from one another, do appear at the same time, we can, by selecting a layer as a basis for identification, recognize the other layers either as the primary wall or as any of the three layers of the secondary wall. Because the surface of a split section of a cell wall has small intermicrofibrillar spaces, the contrast in the replication images is so emphasized that the orientation of microfibrils in the wall can be seen clearly. Therefore, the specimens obtained by the disintegration or the replication method are very useful for observing the fine structure of cell wall. The replicated specimens are especially excellent, in that they can be free from damage by the electron beam.

However, the disintegration and the replication methods could not serve one important purpose, that is, to distinguish the primary wall and the three layers of the secondary from one another and to indicate their thickness. It was necessary, therefore, to take up again the ultrathin transverse sectioning of tracheids. And fortunately it was possible, owing to the

great improvements in ultramicrotomes and knives in recent years, to get excellent ultrathin sections almost continually. Thin sections of a high polymer substance such as a wood cell wall are, in electron microscopy, subject to insufficiency of image contrast and to damage by the electron beam. However, these inconveniences can be overcome by removing embedding materials from the sections and by shadowing procedures. The structure of the primary wall and of the layers of the secondary wall can be clarified by comparing the micrographs of sections of tracheids having incrusting substances removed by delignification with those of untreated tracheids.

One result of the above-mentioned electron microscopic observations, based primarily on the disintegration, replication and ultrathin sectioning methods, is that the physical organization of tracheid walls, already suggested in the investigations by optical microscopy and X-ray diffraction, was confirmed. Another is that we could discover hitherto unknown structures, such as a crossed and an intermediate structure in the secondary wall, and a wart structure in tracheids of some species.

Some representative electron micrographs will be referred to in the following discussion. (The abbreviations P, $S_1$, $S_2$, and $S_3$ stand, respectively, for the primary wall and the outer, the central, and the inner layer of the secondary wall.)

*Distinction of the Primary Wall and the Three Layers of the Secondary Wall.* In the electron micrographs of the transverse sections of tracheids we can, owing to the difference of penetrating power of the electron beam and to surface contrast, distinguish three different layers inside the intercellular layer (Fig. 1) (Yamamoto *et al.*, 1956; Frei *et al.*, 1957; Harada, 1958; Jayme and Fengel, 1961a, b; Wardrop, 1963, 1964). By comparing the micrographs of untreated with delignified sections, these three layers can be identified as the P + $S_1$, the $S_2$, and the $S_3$, and the existence of a wart structure (W) inside them can also be observed. The wall P can be distinguished clearly from the layer $S_1$ in the delignified section (Fig. 2), though not in the untreated section. This is probably because the microfibrils in the wall P are filled with incrusting substances, such as lignin. We cannot measure the thickness of the Wall P and the layers $S_1$, $S_2$, and $S_3$ because a delignified tracheid, when embedded in methacrylate, swells markedly, though we can measure their thickness in the enlarged micrographs of the untreated section (Fig. 3). According to measurements of an earlywood tracheid of *Pinus densiflora*, the wall P is 0.06 $\mu$ in thickness; the layer $S_1$, 0.31 $\mu$; the layer $S_2$, 1.93 $\mu$; and the layer $S_3$, 0.17 $\mu$; the layer $S_2$ occupying about 78% of the whole wall in thickness. This result is almost the same as that obtained by Jayme and Fengel (1961a) for spruce. A lamellar structure in the layers $S_1$, $S_2$, and $S_3$, and a wart layer along

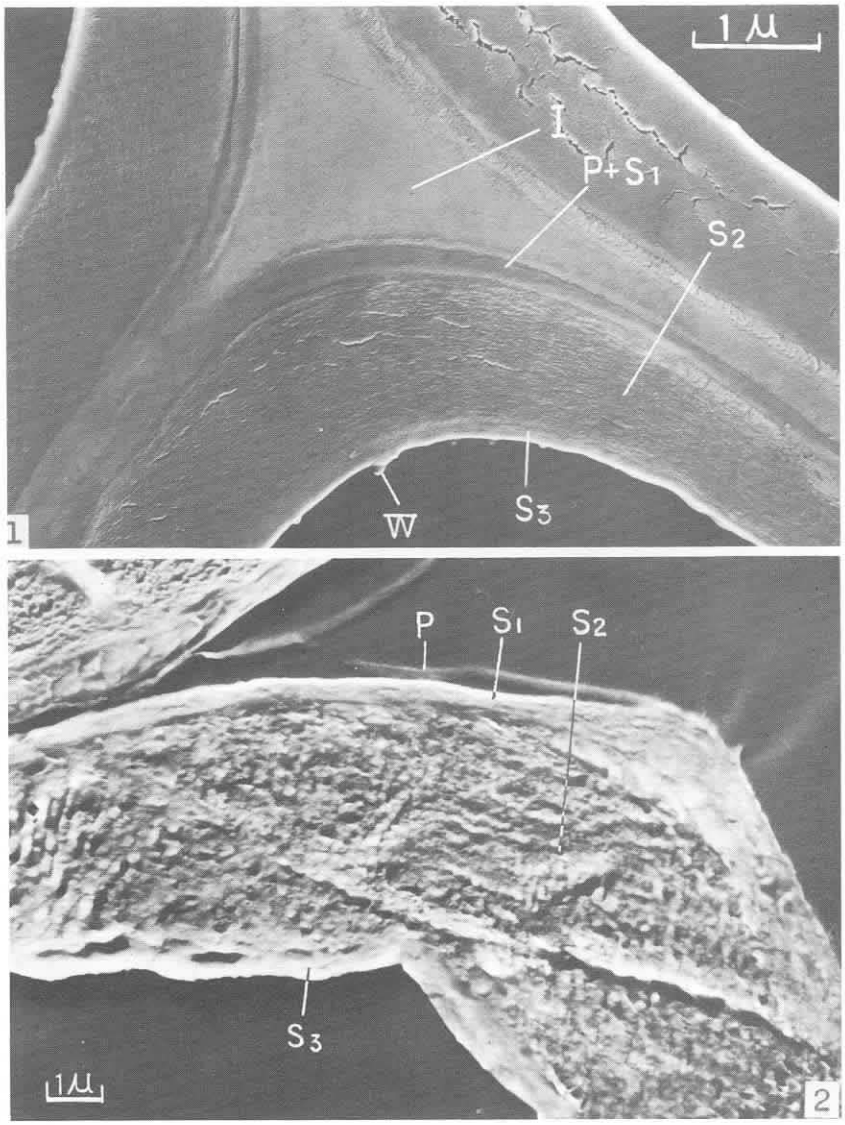



FIG. 1. *Pinus densiflora*. A transverse section showing the three layers of the secondary wall in earlywood tracheids.

FIG. 2. *Pinus densiflora*. A transverse section of delignified tracheids showing the primary wall and the three layers in the secondary wall.

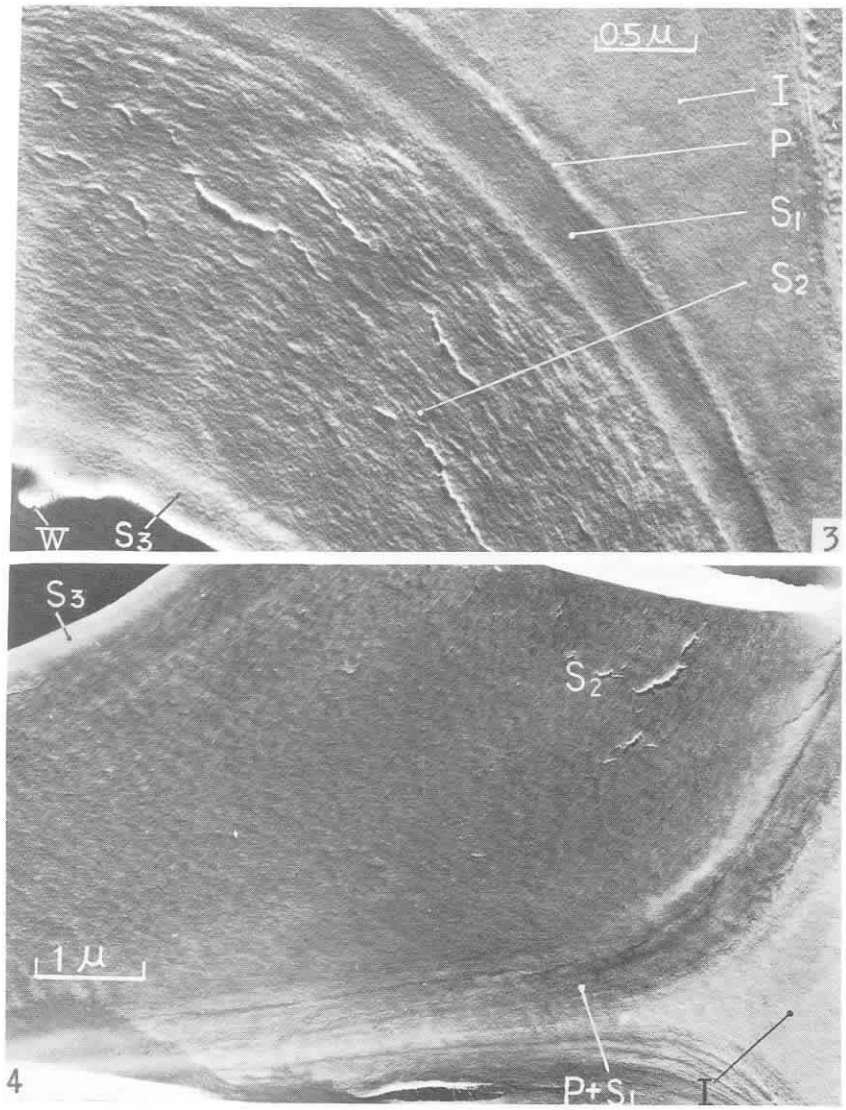



FIG. 3. *Pinus densiflora.* A transverse section of an earlywood tracheid showing the intercellular layer (I), the primary wall (P), the three layers ($S_1$, $S_2$, $S_3$) of the secondary wall and the wart structure (W).

FIG. 4. *Pinus densiflora.* A transverse section of a latewood tracheid showing the individual layers of a cell wall.

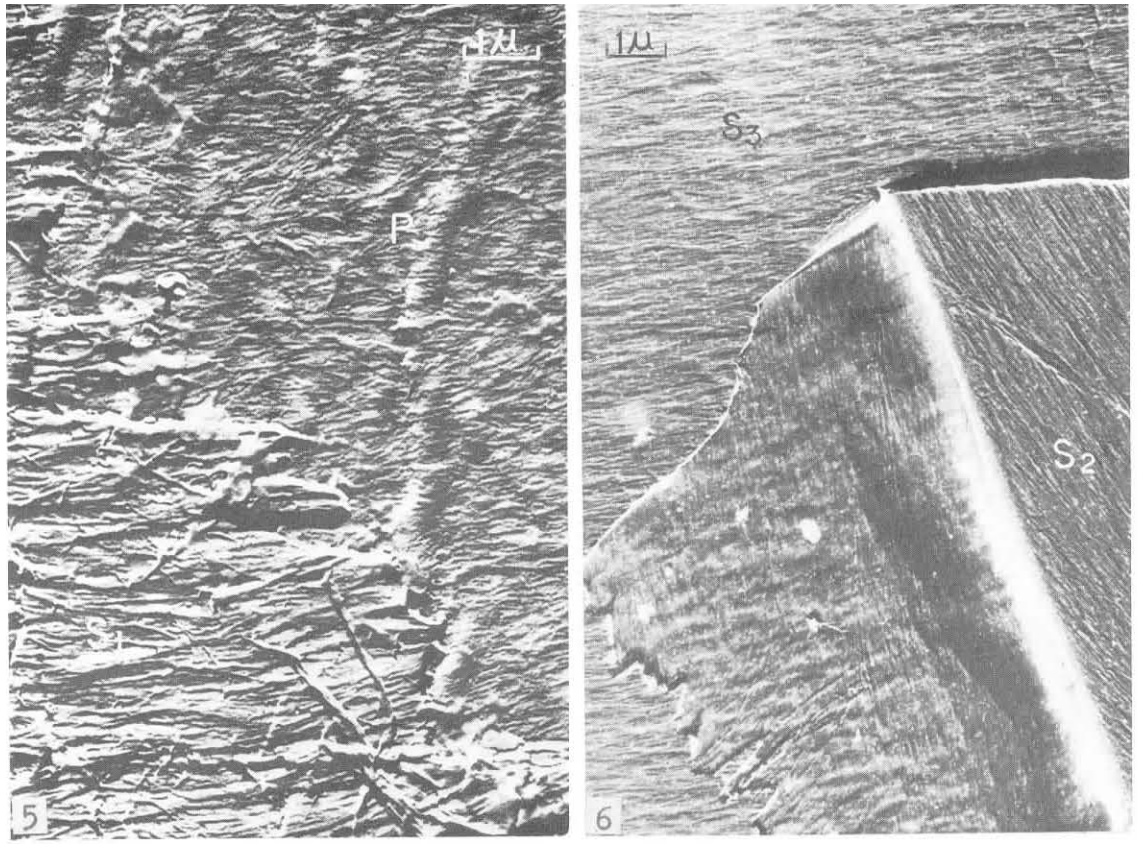



FIG. 5. *Picea jezoensis*. A replica of an earlywood tracheid showing microfibrillar orientation in P and $S_1$. *Note*: the major cell axis in Figures 5 through 15 is vertical (↕). In Figures 16, 17, and 22, the major cell axis is horizontal (↔).
FIG. 6. *Picea jezoensis*. A replica of an earlywood tracheid showing the microfibrillar orientation in $S_2$ and $S_3$.

the lumen were also observed. Cell walls of a latewood tracheid are far thicker than those of an earlywood one, probably because the layer $S_2$ of the former is much thickened (Fig. 4).

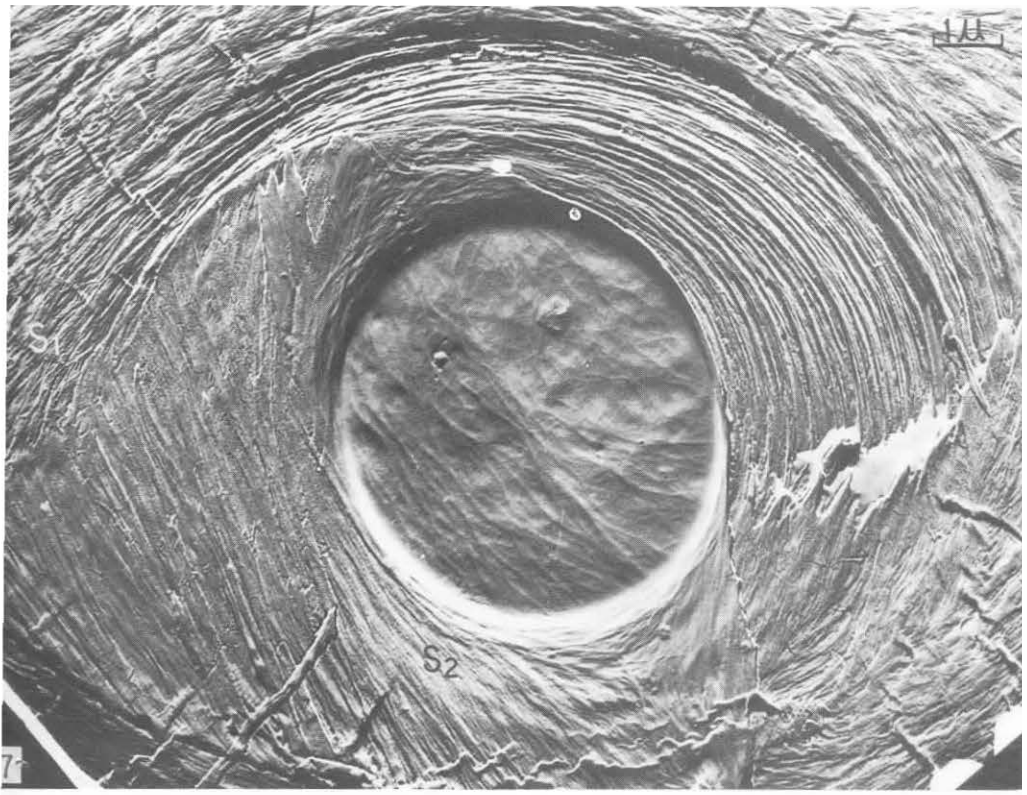
*Microfibrillar Orientation in the Primary and the Secondary Walls.* From the micrographs of replicas of a surface of a chemically macerated tracheid, a net-like orientation of microfibrils in the wall P was confirmed (Kobayashi and Utsumi, 1951). According to the micrographs of disintegrated and replicated specimens of a cambial cell, the wall P is composed of two layers ($P_1$ and $P_2$) different from each other in their orientation of microfibrils (Wardrop, 1964). The structure of the wall P is also observed in the micrographs of replicas of an untreated tracheid, and it is assumed that the openings in the wall P are more completely filled with incrusting substances than those in the layer $S_1$ (Fig. 5). As to the microfibrillar orientation in the layers $S_1$, $S_2$, and $S_3$, many workers have confirmed the earlier findings of polarizing microscopy through the use of electron microscopy (Kobayashi and Utsumi, 1951, 1955; Liese and Fahnenbrock, 1952; Harada and Miyazaki, 1952; Harada *et al.*, 1958; Liese, 1963). The microfibrils in the layer $S_1$ are oriented almost to a right angle to the tracheid axis, following a flat helical pattern (Fig. 5), while those in the layer $S_2$ show a steep helical structure (Fig. 6), oriented at an angle of 20 to 30° to the axis in the case of earlywood tracheids of *Cryptomeria japonica* and *Picea jezoensis*, and of 5 to 10° in the case of a latewood tracheids of the same species. In the layer $S_3$ (Fig. 6), the microfibrils follow a flat helical pattern as in the $S_1$. It is said that a spruce tracheid sometimes lacks the layer $S_3$, but at least in *Picea jezoensis* there exists the $S_3$ with a flat helical structure (Fig. 6).

The assumption by Bailey and Vestal (1937a, b) that the arrangement of microfibrils is, in the places where there are pits, far more deviated than in the places where there are no pits, was also confirmed by electron microscopic observations. In the pit border, the microfibrils are oriented in concentric circles, while around it the microfibrils of the layer $S_1$, of a flat helical structure, are so oriented as to surround a pit aperture, though they seem to be unconnected with the microfibrils in the pit border. Therefore, there is some doubt that the pit border region belongs to the $S_1$ (Fig. 7). Also in the $S_2$ and the $S_3$, the microfibrils are so oriented as to avoid the pit aperture (Figs. 7 and 8). When a half-bordered pit pair

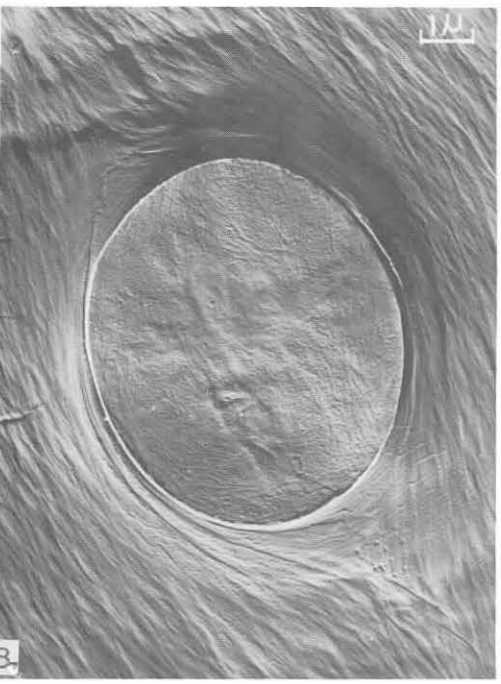
FIG. 7. *Picea jezoensis*. A replica of an earlywood tracheid showing the microfibrillar orientation of $S_1$ and $S_2$ in the pit region.

FIG. 8. *Pinus densiflora*. A replica of an earlywood tracheid showing the microfibrillar orientation of $S_2$ in the pit region.

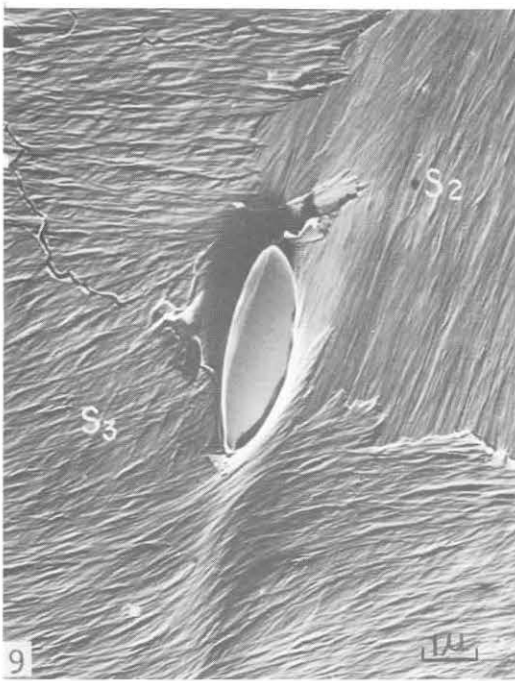
FIG. 9. *Picea jezoensis*. A replica of an earlywood tracheid showing $S_2$ and $S_3$ in the region of a half-bordered pit.




7



8




9

occurs between a tracheid and a ray parenchyma cell, the pit aperture of the tracheid wall takes the form of a convex lens. The long axis of the pit aperture is almost parallel to the microfibrillar orientation in the layer $S_2$ (Fig. 9).

*Parallelism of Microfibrils in the Secondary Wall.* Even in a lamella of the secondary wall, the microfibrils are not arranged exactly parallel with one another. The degree of parallelism of microfibrils is the greatest in the layer $S_2$, and the least in the $S_3$, being medium in the $S_1$. The comparison of an earlywood tracheid with a latewood one, as to the $S_2$, the thickest layer of the secondary wall, shows that the perfection of microfibrillar parallelism is greater in the latter than in the former (Figs. 10 and 11). However, even in the latewood tracheids the microfibrils of the $S_2$ are not perfectly parallel with one another (Harada *et al.*, 1958; Wardrop, 1964).

*Crossed and Intermediate Structures.* Optical mocroscopic investigations could not demonstrate the existence, in the layers $S_1$, $S_2$, and $S_3$, of lamellae where the orientation of microfibrils is crossed. Electron microscopy, however, revealed at least two such lamellae in the $S_1$ (Wardrop, 1954; Meier, 1955; Frei *et al.*, 1957). Wardrop (1957) even suggests that the $S_1$ of a tracheid of *Pinus radiata* has a structure that consists of three patterns of microfibril orientation: a fine grid pattern, a coarse grid pattern, and a complete. However, such a crossed lamellar structure in the $S_1$ must be considered in relation to the structures of other layers: namely, a flat structure in the $S_1$, and an intermediate one between the $S_1$ and the $S_2$.

The existence, between the $S_1$ and the $S_2$, or between the $S_2$ and the $S_3$, of an intermediate structure, in which the angle of microfibrils changes gradually until the direction of their orientation becomes reversed, has been unknown till recently. Kobayashi and Utsumi (1955) suggested, on assuming the existence of microlamellae as wide as microfibrils, that all the layers of the secondary wall are of a minute obliquely crossed structure. Harada *et al.*, (1958), on applying electron microscopy to the replicas of longitudinal sections cut with a microtome, of *Picea jezoensis* and *Pinus densiflora*, examined the intermediate structure in the secondary wall, taking an already known layer as a standard. The layers $S_1$, $S_2$, and $S_3$ are each composed of several lamellae, and the angles and directions of the microfibrillar orientation in these lamellae are almost the same. Between the $S_1$ and the $S_2$, however, or between the $S_2$ and $S_3$, there can be seen some lamellae where the angle of microfibrils changes gradually, and not, as in the above-mentioned patterns, suddenly, until the direction of their orientation becomes quite reversed (Figs. 12, 13, and 14). Though

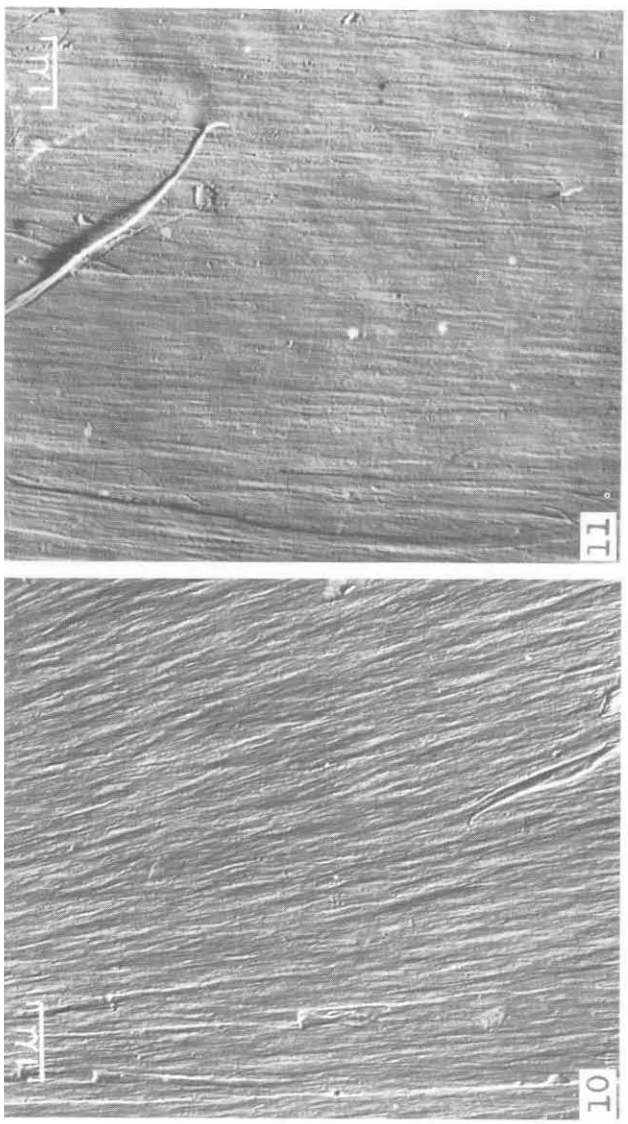
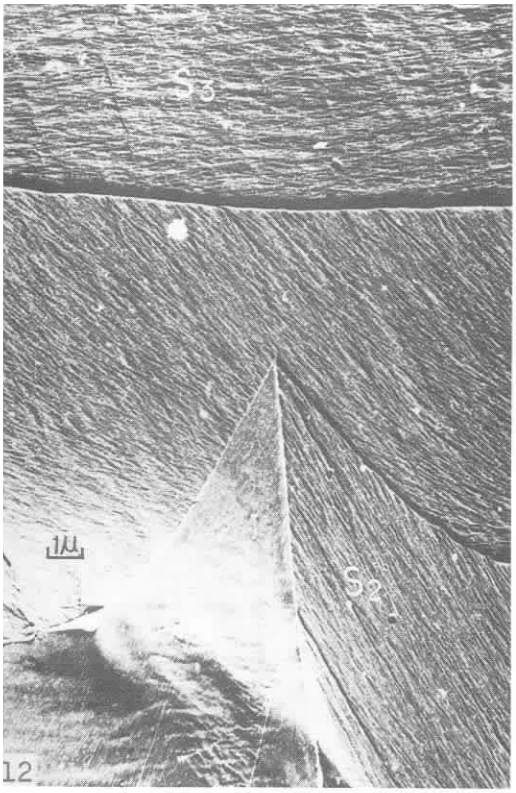
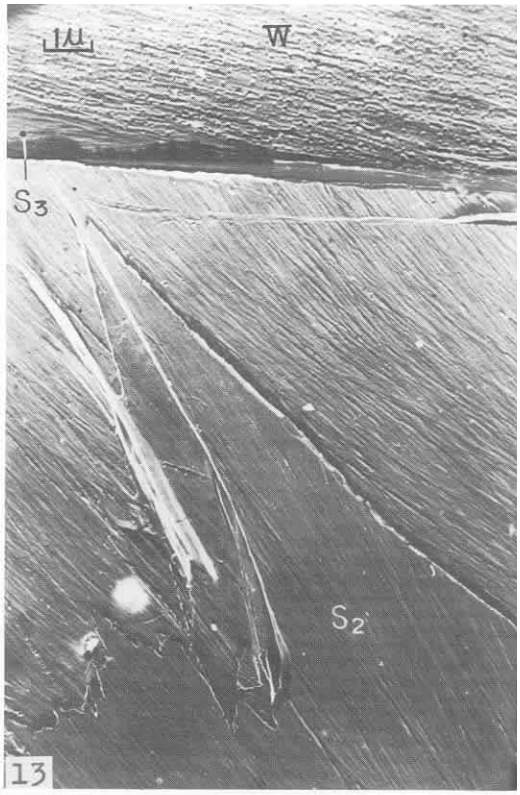


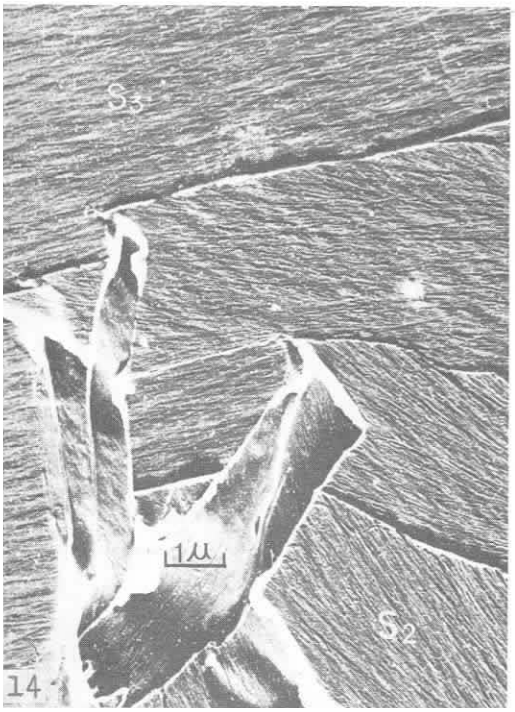

FIG. 10. *Cryptomeria japonica*. A replica of an earlywood tracheid showing the microfibrils in $S_2$. FIG. 11. *Cryptomeria japonica*. A replica of a latewood tracheid showing the microfibrils in $S_2$.



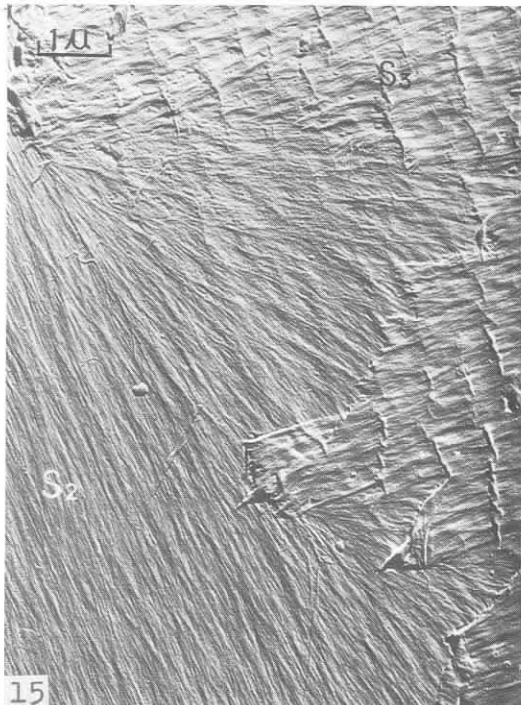
12



13



14



15

it is possible to regard such a gradual change of the angle of the microfibrillar orientation as attributable to a very few lamellae, at any rate in this case it can be seen that the microfibrils change the direction of their orientation in a form like a spread fan, between the $S_2$ and $S_3$ (Fig. 15). Incidentally, it may be that the raised band observed between the $S_1$ and the $S_2$, or between the $S_2$ and the $S_3$, in the electron micrographs of the transverse sections of a tracheid (cf. Fig. 3), suggests the existence of an intermediate structure there.

## ORGANIZATION OF RAY PARENCHYMA CELLS

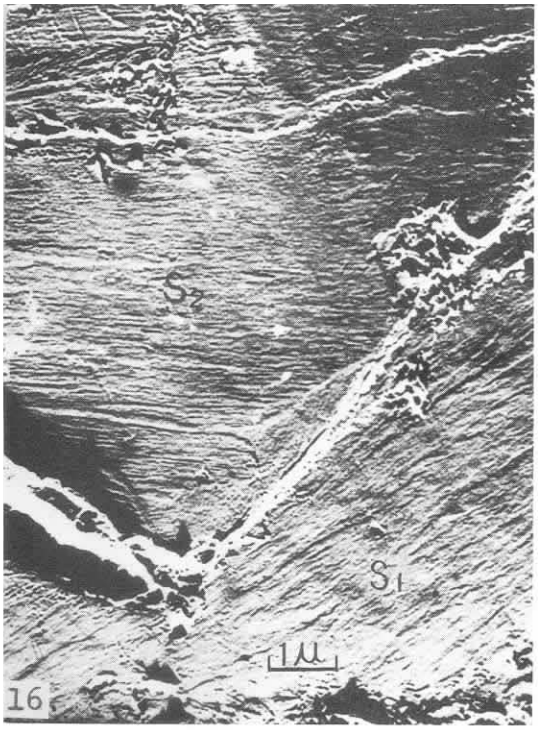
In softwood, ray parenchyma cells constitute only 2 to 4% of the wood volume, and therefore they have not been investigated as much as tracheid walls. It was found with the aid of the polarizing microscope that the micelles of ray parenchyma cells are oriented spirally, and that the primary wall and the layers of the secondary wall are distinguishable (Wardrop and Dadswell, 1952). Not only the physical organization of parenchyma cells of *Cryptomeria japonica*, but also the distribution of their chemical composition has been minutely examined by Harada and Wardrop (1960). Between crossed nicols of the polarizing microscope it can be observed that ray parenchyma cells are divided into three layers: the thin central layer ($S_2$), and the comparatively thick outer ($S_1$) and inner ($S_3$) layers. By measuring birefringence, it is assumed that the micelles in the layers $S_1$ and $S_3$ are oriented in a gentle inclination to the cell axis, while those in the $S_2$ are almost parallel to it. By applying electron microscopy to the replicas of ray parenchyma cells, much information has been obtained: that in the primary wall the microfibrils show a net-like structure such as in tracheid walls, and that in the secondary wall there can be found a layer in which the microfibrils are oriented quite parallel with the cell axis, and two layers in which they are oriented at an angle of 30 to 60° to the axis, the former being placed between the latter (Fig. 16). In collation with the observations by the polarizing microscope, these three layers are assumed to correspond to the layers $S_1$, $S_2$, and $S_3$. The existence, along the lumen side, of a layer in which the microfibrils are arranged irregularly, was discovered for the first time (Fig. 17). In tracheids of *Cryptomeria japonica*, a wart structure can be found, but not in ray parenchyma cells (Fig. 17).

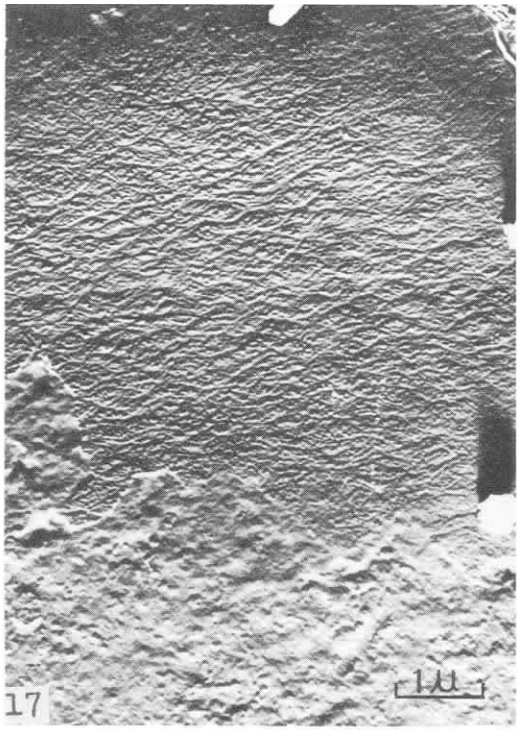
FIG. 12. *Picea jezoensis*. A replica of an earlywood tracheid showing the intermediate structure between $S_2$ and $S_3$.

FIG. 13. *Pinus densiflora*. A replica of an earlywood tracheid showing the same structure as shown in Fig. 12. W—wart layer.

FIG. 14. *Picea jezoensis*. A replica of an earlywood tracheid showing the intermediate structure between $S_2$ and $S_3$.

FIG. 15. *Picea jezoensis*. A replica of an earlywood tracheid showing the intermediate structure between $S_2$ and $S_3$.



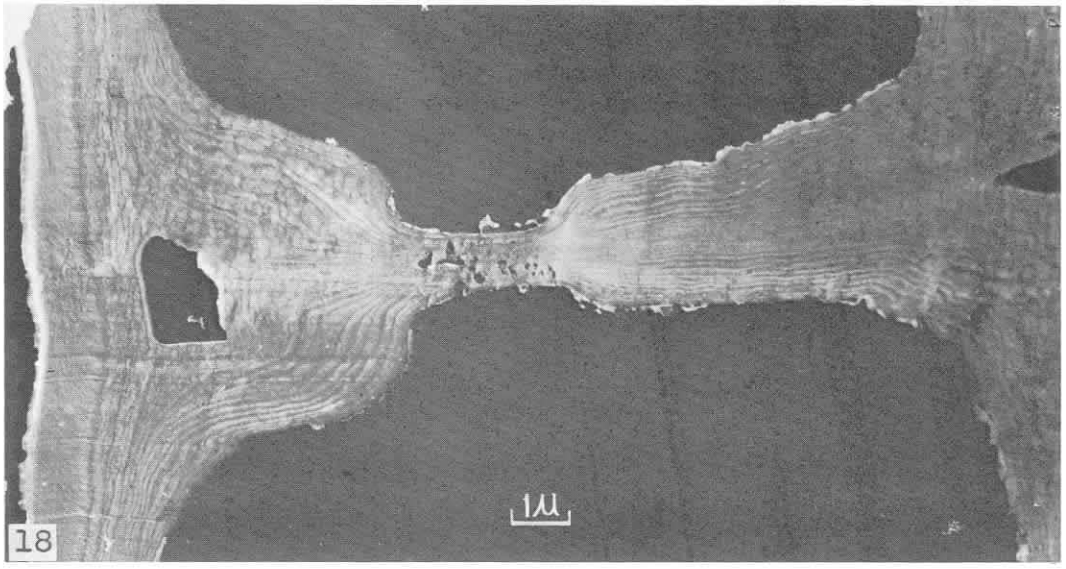




FIG. 16. *Cryptomeria japonica.* A replica of a ray parenchyma cell showing $S_1$ and $S_2$ (Harada and Wardrop, 1960). FIG. 17. *Cryptomeria japonica.* A replica of a ray parenchyma cell showing the net-like structure of microfibrils on the lumen side (Harada and Wardrop, 1960). FIG. 18. *Cryptomeria japonica.* A transverse section of ray parenchyma cells showing the lamellae in the cell wall.

The existence of an intermediate structure in the secondary wall, as in the case of a tracheid, is a matter for further investigation.

In the electron micrographs of the ultrathin sections of ray parenchyma cells, very distinct lamellae can be found (Fig. 18), but the distinction of the layers, such as the P, $S_1$, $S_2$, and $S_3$ in tracheid walls, cannot be observed, nor can any intercellular layer be distinguished between the ray parenchyma cells. This is probably because lignin is equally distributed throughout the whole cell wall (Fig. 19), as was suggested by the results

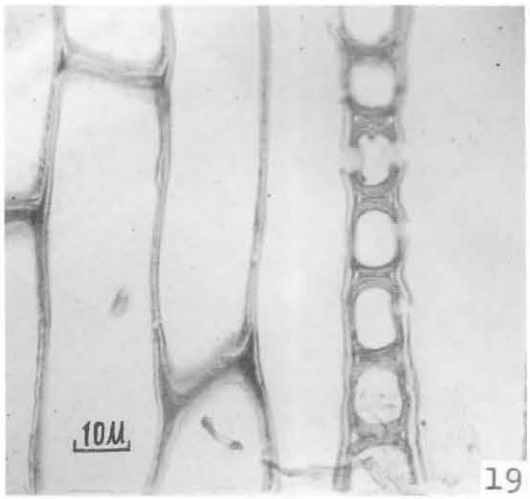
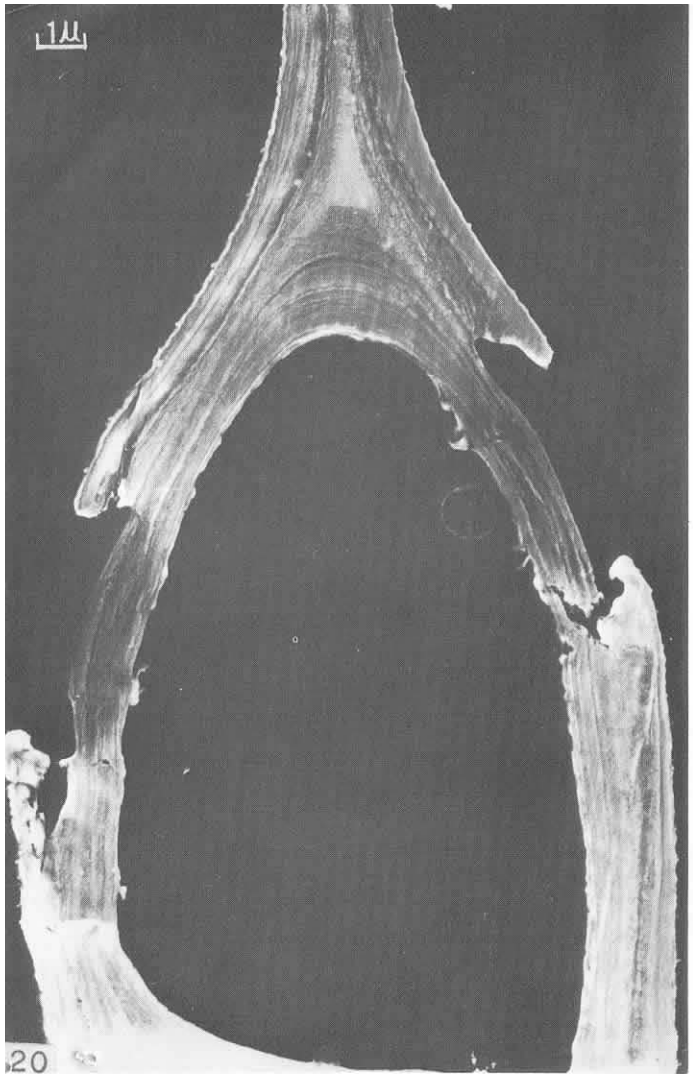


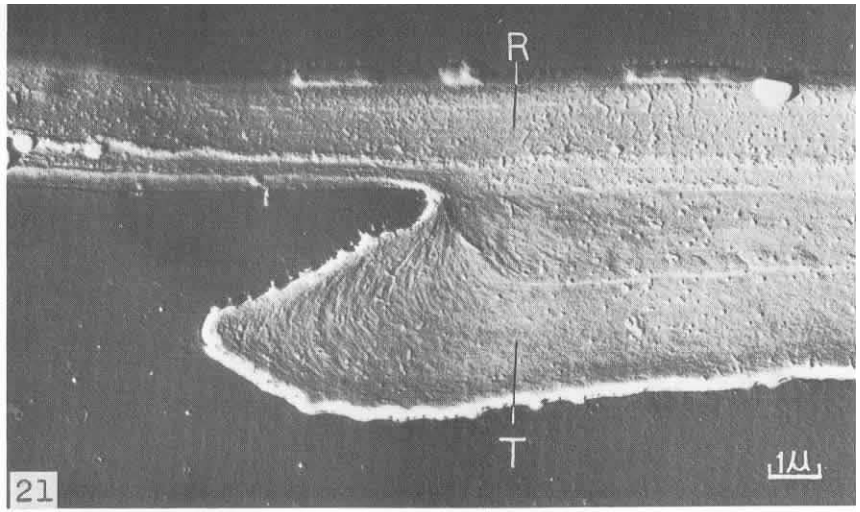

FIG. 19. *Cryptomeria japonica*. An ultraviolet micrograph of a transverse section of ray parenchyma cells showing the distribution of lignin in the cell wall (Harada and Wardrop, 1960).

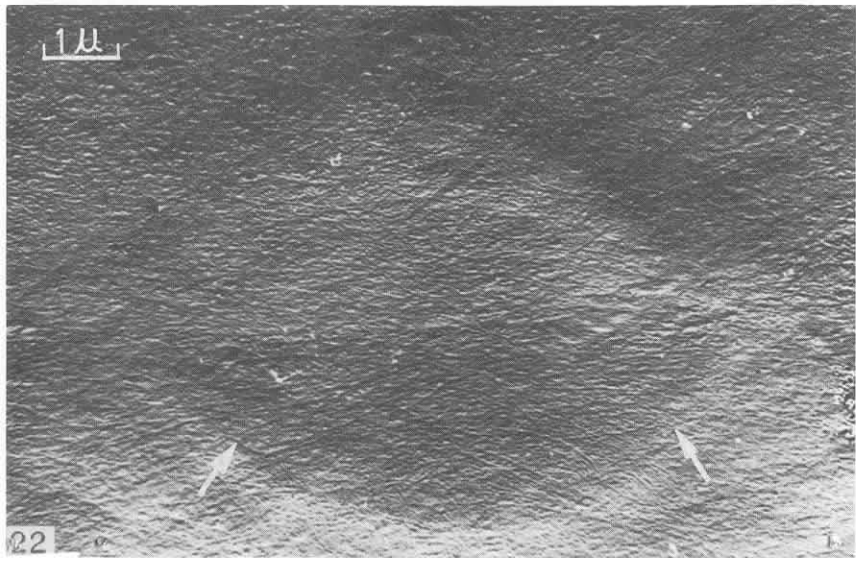
of the investigation by Asunmaa and Steenberg (1957) on the scattering density of the intercellular layer between tracheids.

It is assumed that the pit membrane in a half-bordered pit pair, formed between a ray parenchyma cells and an axial tracheid, consists of an intercellular layer and the two primary walls. However, as shown in Figure 20, a transverse section of a ray parenchyma cell of *Cryptomeria japonica*, the aperture of a simple pit of a parenchyma cell, which should be open, is completely blocked by the cell wall (Fig. 21) (Harada, 1964).

This feature has been also demonstrated by the replica micrograph taken from the lumen side of the ray parenchyma cell (Fig. 22). Though this has been observed also in the parenchyma cells of *Thuja plicata* (Krahmer and Côté, 1963), it is a matter for further investigation whether this is universal in all softwood species.


1μ
20


R
T
1μ
21


1μ
22

## REFERENCES

Asunmaa, S., and Steenberg, G. (1957) "Relative scattering densities in pine heartwood, pine sapwood and spruce." *Svensk Papperstid.* 60:751–761.

Bailey, I. W., and Kerr, T. (1935) "The visible structure of the secondary wall and its significance in physical and chemical investigations of tracheary cells and fibers." *J. Arnold Arboretum* 16:273–300.

———, and Vestal, M. R. (1937a) "The orientation of cellulose in the secondary wall of tracheary cells." *J. Arnold Arboretum* 18:185–195.

——— and ——— (1937b) "The significance of certain wood destroying fungi in the study of the enzymatic hydrolysis of cellulose." *J. Arnold Arboretum* 18:196–205.

Bucher, H. (1957) "Die Tertiärwand von Holzfasern und ihre Erscheinungsformen bei Coniferen." Holzforschung 11:1–16.

Côté, W. A., Jr., and Day, A. C. (1962) "The G layer in gelatinous fibers—Electron microscopic studies." *Forest Prod. J.* 12:333–338.

Frei, E., Preston, R. D., and Ripley, G. W. (1957) "The fine structure of the walls of conifer tracheids. VI. Electron microscope investigations of sections." *J. Exp. Botany* 8:139–146.

Frey-Wyssling, A. (1954) "The fine structure of cellulose microfibrils." *Science* 119:80–82.

——— (1955) "On the crystal structure of cellulose." *Biochim. Biophys. Acta* 18:166–169.

———, Mühlethaler, K., and Wyckoff, R. W. G. (1948) "Mikrofibrillenbau der Pflanzlichen Zellwand." *Experientia* 4:475.

Harada, H. (1958) "Electron microscopic structure of wood" (in Japanese). *J. Kobunshi* 7:615–619.

——— (1964) In press.

———, Kishima, T., and Kadita, S. (1951) "The micellar orientation in the secondary wall of coniferous tracheids (II)." Bulletin of the Wood Research Institute, Kyoto Univ., Japan, No. 6, pp. 34–42.

———, and Miyazaki, Y. (1952) "The electron-microscopic observation of the cell wall of conifer tracheids." *J. Japan. Forestry Soc.* 34:350–352.

———, ———, and Wakashima, T. (1958) "Electron microscopic investigation on the cell wall structure of wood." Bulletin of the Govt. Forest Experiment Station, No. 104 (Meguro, Tokyo, Japan).

———, and Wardrop, A. B. (1960) "Cell wall structure of ray parenchyma cells of a softwood (Cryptomeria japonica)." *J. Japan Wood Res. Soc.* 6:34–41.

Hengstenberg, J., and Mark, H. (1928) "Rötgenuntersuchungen über den Bau der C-Ketten in Kohlenwasserstoffen." *Z. Krist.* 67:583–594.

Hodge, A. J., and Wardrop, A. B. (1950) "An electron microscopic investigation of the cell wall organization of conifer tracheids and conifer cambium." *Australian J. Sci. Res.*, B 3:265–269.

Jayme, G., and Fengel, D. (1961a) "Beitrag zur Kenntnis des Feinbaus der Frühholztrache-

---

FIG. 20. *Cryptomeria japonica*. A section of wood showing the half-bordered pit pair between an axial tracheid and a ray parenchyma cell.

FIG. 21. *Cryptomeria japonica*. A section of a ray parenchyma cell (R) and an axial tracheid (T) showing the same structure as shown in Fig. 20.

FIG. 22. *Cryptomeria japonica*. A replica of a ray parenchyma cell showing the closure of the simple pit aperture with the parenchyma cell wall itself. (Arrows show the outline of the margin of the bordered pit.)

iden. Beobachtung an Ultradünnschnitten von Fichtenholz." *Holz Roh- Werkstoff* 19:50–55.

_____ and _____ (1961b) "Beitrag zur Kenntnis des Feinbaus der Fichtenholztracheiden. II. Beobachtung an Ultradünnschnitten von delignifiziertem Holz und Ligningerüsten." *Holzforschung* 15:97–102.

_____, and Koburg, E. (1959) "Über den elektronenoptisch bestimmten Durchmesser der Mikrofibrillen von Laubholzzellelementen." *Holzforschung* 13:37–43.

Kerr, T., and Bailey, I. W. (1934) "The cambium and its derivative tissues. No. X. Structure, optical properties and chemical composition of the so-called middle lamella." *J. Arnold Arboretum* 15:327–349.

Kobayashi, K., and Utsumi, N. (1951) "Electronmicroscopy of conifer tracheids (in Japanese). Committee Note on Electron Microscopy, No. 56, p. 93.

_____ and _____ (1955) "Reformation of cellulose micell and microfibrils." *Seni Kenkyusho Koenshu*, No. 12, pp. 159–187.

Krahmer, R. L., and Côté, W. A., Jr. (1963) "Changes in coniferous wood cells associated with heartwood formation." *Tappi* 46:42–49.

Kubo, T., and Go, Y. (1941) "Study on the fine structure of wood by X-ray diffraction." *J. Soc. Chem. Ind. (Japan)* 44:651–655.

Liese, W. (1963) "Tertiary wall and warty layer in wood cells." *J. Polymer Sci.*, Part C 2:213–219.

_____, and Fahnenbrock, M. (1952) "Elektronmikroskopische Untersuchungen über den Bau der Hoftüpfel." *Holz Roh- Werkstoff* 10:197–201.

Meier, H. (1955) "Über den Zellwandbau durch Holzvermorschungspilze und die submikroskopische Struktur von Fichtentracheiden und Birkenholzfasern." *Holz Roh-Werkstoff* 13:323–338.

Mühlethaler, K. (1960) "Die Feinstruktur der Zellulosemikrofibrillen. *Z. Schweiz. Forstv.* 30:55–65.

Ohara, K. (1939) "Relation between the species of conifers and the micell angle of their tracheids wall" (in Japanese). *Jinkenkai* 7:635–637.

Onaka, F. (1936) "Identification of olden woods from ancient tombs" (in Japanese). *J. Japan Forestry Soc.* 18:588–602.

_____, and Harada, H. (1951) "The micellar orientation in the secondary wall of coniferous tracheids." *J. Japan Forestry Soc.* 33:60–64.

Preston, R. D. (1934) "The organization of the cell wall of the conifer tracheid." *Phil. Trans. Roy. Soc. London, Ser. B* 224:131–172.

_____ (1952) *The Molecular Architecture in Plant Cell Wall.* Chapman and Hall, London.

Rånby, B. G. (1958) "The fine structure of cellulose fibrils." In *Fundamentals papermaking Fibers, Trans. Symp. Cambridge, Engl., 1957* (F. Bolam, ed.), pp. 1–27.

_____ (1957) "The organization and properties of the outer layer of the secondary wall in

Wardrop, A. B. (1954) "The fine structure of the conifer tracheid." *Holzforschung* 8:12–29.

_____ (1957) "The organization and properties of the outer layer of the secondary wall in conifer tracheids." *Holzforschung* 11:102–110.

_____ (1963) "Morphological factors involved in the pulping and beating of wood fibers." *Svensk Papperstid.* 66:231–247.

_____ (1964) "The structure and formation of the cell wall in xylem." In *The Formation of Wood in Forest Trees* (M. H. Zimmerman, ed.), pp. 87–134. Academic Press, N.Y.

_____, and Dadswell, H. E. (1952) "The cell wall structure of xylem parenchyma." *Australian J. Sci. Res., Ser. B*—Bio. Sci. 5:223–236.

———, and Preston, R. D. (1947) "The submicroscopic organization of the cell wall in conifer tracheids and wood fibers." *J. Exp. Botany* 11:20–30.

Williams, R. C., and Wyckoff, R. W. G. (1946) "Applications of metallic shadow casting to microscopy." *J. Appl. Phys.* 17:23–33.

Yamamoto, T., Kadita, S., and Kobayashi, K. (1956) "Electron microscopy of ultrathin sections of softwood" (in Japanese). *Trans. Kansai-Branch Japan. Forestry Soc.*, pp. 161–162.